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TITLE: In Vivo Imaging of Cortical Inflammation and Subpial Pathology in Multiple Sclerosis by Combined PET and MRI

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14. ABSTRACT
Post-mortem studies in multiple sclerosis (MS) suggested that cortical demyelinating lesions, which are hardly detected in vivo on conventional magnetic resonance imaging (MRI) scans, are an important correlate of disability, and are driven by organized neuroinflammation with the activation of microglia. Activated microglia upregulate expression of the 18kDa translocator protein (TSPO), which can be imaged in vivo with [^{11}C]PBR28, a second generation TSPO ligand.
In this study, we combine ultra-high field 7 Tesla (T) MRI, which has demonstrated greater sensitivity to cortical lesions than conventional MRI, with [^{11}C]PBR28 positron emission tomography (PET) imaging of activated microglia to assess whether more severe structural cortical pathology in MS is related to the presence of neuroinflammation.
Our initial findings show that high-resolution [^{11}C]-PBR28 PET imaging is able to detect in vivo diffuse inflammation in different brain tissue compartments in MS, particularly in cortex and cortical sulci. Additionally, the degree of inflammation in cortical sulci is associated with neurological disability, suggesting that this pattern of cortical disease can be the pathological basis for disease progression in many MS cases.

15. SUBJECT TERMS
Multiple sclerosis; cortex; cortical sulci; neuroinflammation; microglia; cortical demyelination; ^{11}C]PBR28 PET imaging; ultra-high field MRI; neurological disability.

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1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disorder of the central nervous system (CNS), and the leading cause of non-traumatic disability in young adults in Western countries. Histopathological examinations of MS brains indicate that cortical demyelinating lesions are potential biomarkers of MS progression.

Since cortical lesions appeared topographically related to focal meningeal inflammation in some pathological studies, it has been hypothesized that cortical demyelination in MS may be driven by organized meningeal inflammation through the activation of microglia, accompanied by a decreasing gradient of demyelination away from the pial surface. Histopathological evidence that the cortex can be the site of inflammatory demyelinating lesions near the time of MS onset further supports the existence of an early pathological process that primarily targets the cortex, independently from white matter (WM).

Although largely undetected on conventional magnetic resonance imaging (MRI) scans, cortical lesions, including the subpial type, have been imaged in vivo with improved sensitivity and spatial specificity at ultra high-field 7 Tesla (T) MRI. We previously demonstrated that surface-based mapping of quantitative T_2* (q-T_2*) as a function of cortical depth from ultra high-resolution gradient echo 7 T MRI images is highly reproducible, and could prove useful for studying the laminar architecture of the cortex in vivo, and for characterizing cortical pathological abnormalities in MS associated with changes in cortical myelin and/or iron concentration.

The purpose of this project is to evaluate inflammation and structural tissue changes in the cortex of patients with relapsing-remitting (RR) MS by combining advanced MR studies at 7 T MRI, to measure cortical lesions and diffuse subpial pathology, with positron emission tomography (PET) using the 18kDa translocator protein (TSPO)-targeting radioligand [^{11}C]-PBR28 to directly quantify microglia activation.

The overall working hypothesis is that patients with MS will show widespread cortical inflammation, which is topographically associated with the presence of structural cortical abnormalities (lesions), as suggested by post-mortem studies. The ability to quantify in vivo MR tissue changes at different cortical depths from the pial surface, across the whole cortical mantle, and to couple these measurements with assessment of neuroinflammation, can provide insights on the biological basis of cortical degeneration in MS. This, in turn, could help to predict aggressive forms of the disease that can be susceptible to earlier and more specific therapies.

The in vivo study of the inflammatory and degenerative components of cortical disease in MS can have major implications for diagnosing, and understanding the pathogenesis of disease progression in MS.

2. Keywords

1. Multiple sclerosis
2. Relapsing-remitting
3. Cortical lesions
4. Subpial demyelination
5. 7 Tesla
6. Magnetic resonance imaging
7. White matter lesions
8. Normal appearing white matter (NAWM)
9. Positron emission tomography
10. [^{11}C]-PBR28
11. Microglia
12. Marcophages
13. Inflammation
14. Stantard uptake values (SUV)
15. Disability
16. Expanded disability status scale (EDSS)

3. Overall project summary

Current objectives

During Year 1 of the present award we have obtained HRPO approval in February 2014, and initiated afterwards all study procedures related to Aim1 and Aim2 of the SOW.

The overall goal of Aim1 is to "assess in patients with relapsing-remitting multiple sclerosis (RRMS) microglia activation in the cortex, as measured by [^{11}C]-PBR28 binding potential (BP_{ND}), and its association with subpial pathology and cortical lesions".

The overall goal of Aim2 is to "determine the relationship between cortical inflammation, as measured by cortical [^{11}C]-PBR28 BP_{ND}, disability and cognitive performance in patients with MS".

Progress and accomplishments

For such purpose we screened about 20 potential study participants with RRMS, consented for the study five patients and genotyped them for the Ala147Thr polymorphism in the TSPO gene. Four subjects resulted high or mixed affinity binders (Ala/Ala, Ala/Thr) and were enrolled in subsequent study procedures that include: a) assessment of cortical microglia activation during a 90-min acquisition after injection of [^{11}C]-PBR28 using simultaneously acquired positron emission tomography (PET) and MR imaging (Siemens BrainPET scanner); b) assessment of structural cortical demyelinating lesions and diffuse subpial pathology using ultra-high field 7 Tesla MRI combined with multi-channel radiofrequency technology; c) administration to patients of the 9-hole peg test, the Minimal neuropsychological assessment of MS (MACFIMS), the Expanded Disability Status Scale (EDSS), and the Ambulation Index (AI).

We have preliminary data analyzed from two subjects that have completed the study; the remaining consented patients are in the process of completing study procedures. We are continuing to screen potential study participants and have scheduled consenting and genotyping visits for two additional patients.

We have pooled the analyzed MR-PET and 7 T MRI data from the RRMS subjects of this study with those from a small sample (N=7) from an ongoing study funded by the National MS Society, which is focused on more advanced stages of MS, namely secondary-progressive (SP) MS. All subjects underwent 90 minutes of [^{11}C]-PBR28 on a unique Siemens BrainPet scanner, a dedicated brain avalanche photodiode brain PET scanner that can be operated in the bore of a 3 T whole body MR magnet. MRI anatomical scans were simultaneously collected for Freesurfer reconstruction of cortical surfaces and coregistration of PET and MR modalities. Standard uptake values (SUV) maps were created by averaging PET frames (1.25 mm isotropic voxel size) between 60 - 90 minutes. In patients, lesions were segmented from T2* gradient echo images acquired at ultra-high resolution (0.33 x 0.33 x 1 mm voxel size) at 7 T using a 32-channel coil on a separate day. Patients' brain uptake values of [^{11}C]-PBR28 from different brain tissue compartments including cortical and /or WM lesions, cortical sulci, cortical gyri, as well as normal appearing white matter (NAWM) were compared from SUVs from cortex and WM from nine healthy controls (HC), matched for age, gender and binding affinity type.

In patients we also assessed the relationship between cortical inflammation, as measured by cortical [^{11}C]-PBR28 uptake, and neurological disability as measured by EDSS.

Findings from these initial data have been recently presented at the joint meeting of the American and European Committees for Research on Multiple Sclerosis (Actrims-Ectrims), which was held in Boston September 10th-13th 2014 (Gianni' C, Govindarajan ST, Fan AP, Louapre C, Loggia M, Catana C, Tinelli E, Hooker J, Sloane J, Kinkel RP, Mainero C. [^{11}C]-PBR28 PET imaging detects in vivo inflammation in normal appearing white matter and cortical sulci in multiple sclerosis).
The work was highlighted at the closing session of the meeting.
Main findings are presented below.

Results

Table 1 below shows the percent increase in [^{11}C]-PBR28 standard uptake values (SUV) in 9 subjects with MS relative to 9 HC within different brain tissue compartments in the cortex and in WM.

	WM	**NAWM**	**WM lesions**	**Cortex**	**Cortical sulci**	**Cortical gyri**
MS mean (SD)	-	0.72 (0.2)	0.631 (0.2)	0.79 (0.23)	0.80 (0.25)	0.78 (0.23)
HC mean (SD)	0.55 (0.1)	-	-	0.63 (0.15	0.61 (0.15)	0.64 (0.15)
% Increase (MS vs HC)	-	~32%	~15%	~ 25%	~ 30%	~ 23%

Overall in patients, relative to HC, there was an increase in PBR28 SUV in all tissue compartments assessed. The observed increase, however, reached statistical significance only for whole cortex, cortical sulci, and NAWM as shown in ***Figure 1*** (p=0.048; p= 0.035; p=0.03 respectively as assessed by multi-linear regression models including as age and PBR affinity as covariates of no interest). The lowest uptake was seen in WM lesions.
Interestingly, as opposed to SPMS subjects, in RRMS subjects we did not find any clear overlap between visible cortical and leukocortical lesions on 7 T MRI and areas of increased radiotracer uptake.

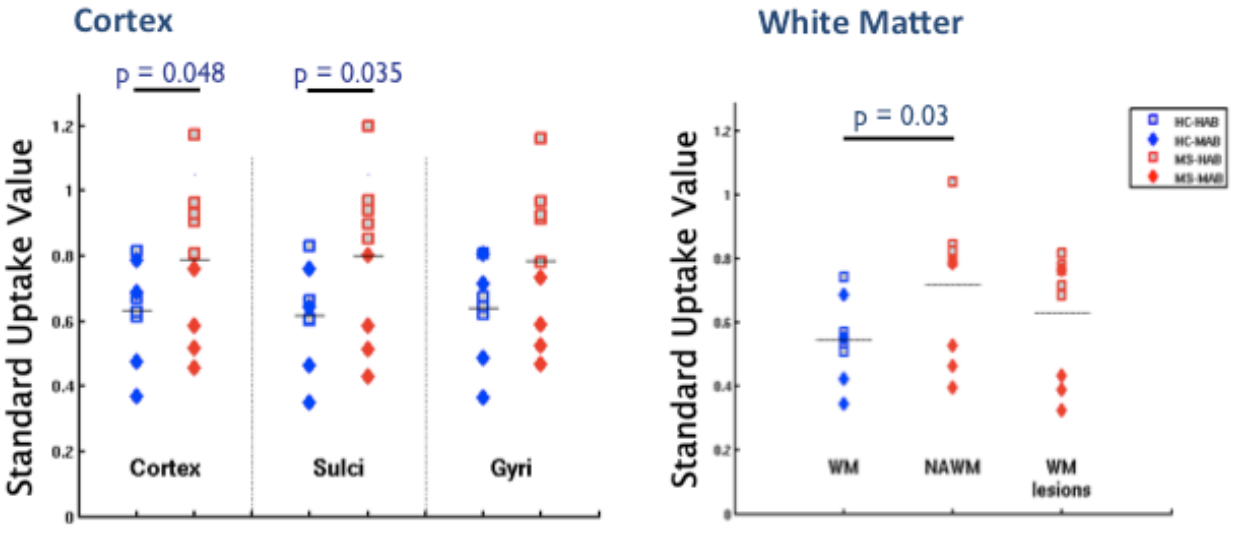

Figure 1. Mean SUV values within different cortical and white matter tissue compartments in individual MS subjects and healthy controls (HC). Horizontal bars indicate group averages. HAB= high affinity binder; MAB= mixed affinity binder.

We also assessed the relationship in the whole group of MS subjects between SUV uptakes in different brain tissue compartments and clinical outcome measures. We found that worse neurological disability as measured by EDSS was associated with increased [^{11}C]-PBR28 SUV in NAWM and cortical sulci (p= 0.01; p=0.04 respectively, by multi-linear regression models corrected for age and PBR affinity, ***Figure 2***).

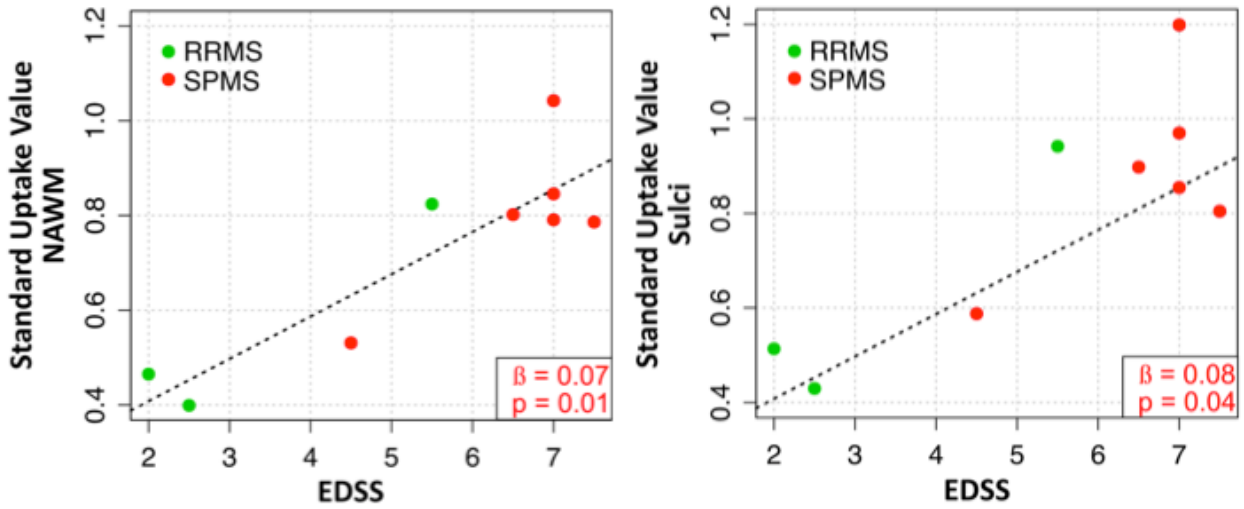

Figure 2. Scatterplots showing a positive correlation in RRMS and SPMS subjects between [^{11}C]-PBR28 standard uptake values (SUV) in normal appearing white matter (NAWM) and cortical sulci and Expanded Disability Status scale (EDSS) score, a measure of neurological disability.

Discussion

Our initial results show diffuse cortical and NAWM inflammation in a small sample of MS patients. Interestingly, only when RRMS subjects from this study were pooled together with patients with more advanced disease stages (SPMS) the results reached statistical significance. This suggests that the diffuse microglia and macrophages activation can be present also in RRMS. With the enrollment of more RRMS patients during Year2 of the present award, we will be able to perform statistics only in these earlier disease stages, and measure the impact of microglia and macrophages activation on clinical outcome measures including cognitive data.

4. Key research accomplishments

A) Our initial findings suggest that high-resolution [^{11}C]-PBR28 MR-PET imaging is able to detect in vivo diffuse inflammation in different brain tissue compartments in MS.

B) We demonstrated for the first time, in vivo, that cortical sulci are involved by extensive microglia activation and that the degree of this inflammation, as measured by [^{11}C]-PBR28 uptake, is associated with neurological disability.

C) Interestingly, as opposed with patients with chronic progressive MS, increased [^{11}C]-PBR28 uptake in RRMS subjects did not seem to overlap with visible lesions on 7 T MRI.

5. Conclusion

High-resolution [^{11}C]-PBR28 MR-PET imaging manifests as a promising tool for assessing in vivo microglia and macrophages activation within different brain tissue compartments in MS, particularly in the cortex and cortical sulci.
Autopsy studies of progressive MS showed that more aggressive cortical pathology was associated with the presence of ectopic meningeal B-cell follicular-like structures that were located along and in the depth of the cerebral sulci (Magliozzi *et al.*, 2007, Magliozzi *et al.*, 2010, Howell *et al.*, 2011), and which are thought to trigger cortical demyelination through the activation of microglia (Lassmann and Lucchinetti, 2008). The role of ectopic meningeal B-cell follicles in early MS has not been elucidated yet.
Using [^{11}C]-PBR28 MR-PET imaging, we found in vivo that in our small MS sample microglia activation was prominent in cortical sulci, in line with the hypothesis that cortical inflammation is a process likely facilitated by the adjacent meningeal inflammatory milieu. The preferential localization of subpial demyelination in cortical sulci can be also explained by the tendency of meningeal inflammatory cells and soluble mediators to collect and concentrate in sulci, while being diluted at the outer gyral brain surface due to physiological flow variations of cerebrospinal fluid (CSF).
The positive correlation between the extent of microglia activation in cortical sulci and neurological disability provides in vivo evidence that this pattern of cortical disease can be the pathological basis for disease progression in many MS cases.
These data need to be confirmed in a larger sample of patients.

6. Publications, Abstracts, and Presentations

1) Gianni' C, Govindarajan ST, Fan AP, Louapre C, Loggia M, Catana C, Tinelli E, Hooker J, Sloane J, Kinkel RP, Mainero C.
[^{11}C]-PBR28 PET imaging detects in vivo inflammation in normal appearing white matter and cortical sulci in multiple sclerosis.
Proceedings of the joint meeting of the American and European Committees for Research on Multiple Sclerosis (Actrims-Ectrims), Boston September 10th-13th 2014.
The work was highlighted at the closing session of the meeting.

7. Inventions, Patents and Licenses

Nothing to report.

8. Reportable Outcomes

Nothing to report.

9. Other Achievements

Nothing to report.

10. References

1) Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain. 2007;130(Pt 4):1089-104.
2) Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, et al. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol. 2010;68(4):477-93.
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4) Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, et al. Inflammatory cortical demyelination in early multiple sclerosis. The New England journal of medicine. 2011;365(23):2188-97.
5) Lassmann H, Lucchinetti CF. Cortical demyelination in CNS inflammatory demyelinating diseases. Neurology. 2008;70(5):332-3.

11. Appendices

Copy of the abstract presented at the Actrims-Ectrims meeting in Boston, September 10th -13th 2014.

Abstract Preview - Step 3/4

- print version -

Topic: Imaging

Keywords: MR-PET, Microglia and Macrophages activation, [11C]PBR28, 7 T MRI

Title: [^{11}C]-PBR28 MR-PET imaging detects in vivo inflammation in normal appearing white matter and cortical sulci in multiple sclerosis

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Text: **Background:** Histopathological studies have implicated activation of microglia and macrophages in both white matter (WM) and cortical lesions pathogenesis in multiple sclerosis (MS), as well as in normal appearing WM (NAWM) diffuse damage. Activated microglia and macrophages upregulate expression of the 18kDa translocator protein (TSPO), which can be imaged in vivo with [^{11}C]PBR28, a second generation TSPO ligand.
Objectives: To assess the presence of activated microglia and macrophages within different lesional and non-lesional brain tissue compartments in MS by combining [^{11}C]PBR28 PET imaging with 7 Tesla (T) MRI.
Methods: Seven MS patients (5 SPMS and 2 RRMS) and 7 healthy controls (HC) matched for age, gender and TSPO affinity binding underwent 90 minutes of [^{11}C]PBR28 imaging on a high resolution Siemens BrainPET scanner. Anatomical MR images were simultaneously acquired for FreeSurfer cortical surface reconstruction and MR-PET image registration. In each subject, standardized uptake value (SUV) maps were created for 60 to 90-minute PET frame (1.25 mm^3 isotropic resolution). Lesions were segmented from T2*-weighted images (0.33×0.33×1 mm^3) acquired on a separate 7T MRI session: WM lesions in all MS subjects; cortical (reaching the pial surface) and leukocortical (extending across GM and WM without reaching the pial surface) lesions in 3 SPMS subjects. Lesion, cortical sulci and gyri masks were co-registered to each [^{11}C]-PBR28 map to extract SUVs. In HC, SUVs were obtained in the cortex (sulci and gyri) and in WM. SUVs were compared between patients and HC using a paired t-test.
Results: Relative to HC, in patients there was a ~24% increase in SUVs (mean±SD) in NAWM (MS=0.711±0.2; HC=0.575±0.1), a ~11% increase in lesional WM (MS=0.641±0.2), ~20% increase in cortical sulci (MS=0.784±0.2; HC=0.653±0.1), and 13% increase in cortical gyri (MS=0.769±0.2; HC=0.679±0.1). In patients, increase in SUVs reached statistical significance relative to HC for NAWM ($p < 0.03$) and cortical sulci ($p < 0.05$). SPMS subjects showed, relative to RRMS, a 17%, 23%, 20% and 14% increase in lesional WM, NAWM, cortical sulci and gyri, respectively. There was no difference between lesional and non-lesional cortical SUVs.
Conclusions: High resolution [^{11}C]PBR28 MR-PET imaging is a promising tool for assessing in vivo microglia and macrophages activation within brain tissue compartments in MS including NAWM and cortical sulci. The latter have been reported as the main location of neuroinflammation and demyelination by post-mortem studies.

Study Support: Claflin Award; NMSS RG 4729A2/1; US Army W81XWH-13-1-0112; FISM Training Fellowship 2012/B/4

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